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The Effect of Neighbors on Gametophyte Development in *Ceratopteris richardii*

AMANDA SAYERS AND ROBERT G. HAMILTON*
Department of Biological Sciences, Mississippi College, Clinton, MS 39058

Previous investigations of gametophyte development have revealed that intergametophytic interactions strongly influence development (e.g., Näf et al., 1975). Studies of the population biology of seed plants have revealed that basic genetic characteristics such as seed size, germination time, and growth rate can influence interactions among neighbors (Silvertown, 1987). Despite the well known effects of neighbors on gametophyte development (Näf et al., 1975) there are few studies of the effects of basic genetic characteristics such as spore size, germination time, and growth rate on gametophyte development.

Schedlbauer (1976) has studied the effects of spore size, germination time, and growth rate on gametophyte development in *Ceratopteris thalictroides* (L.) Brongn. We used methods similar to those of Schedlbauer to investigate the influence of spore size and germination time on gametophyte development in *Ceratopteris richardii* Brongn. Previous investigation has revealed that two distinct developmental states occur among gametophytes developing from multispore cultures of *C. richardii*: small ameristic males and large meristic hermaphrodites (Sayers, unpublished data). Although a small number of vegetative and female gametophytes did occur, they were too rare to be included in any statistical analysis. This investigation thus focussed on large hermaphroditic and small male gametophytes.

MATERIALS AND METHODS

Spores of *C. richardii* were supplied by Thomas Warne of the University of Tennessee at Knoxville. Two treatments, one experimental and one control, were prepared. The control treatment consisted of 30 single spores, each sown onto a 60 mm × 15 mm plastic nutrient agar plate. The experimental treatment consisted of 30 pairs of spores, each sown onto such a plate. Nutrient agar was prepared as described in Klekowski (1969), except that a 1.5% agar concentration was used to reduce the rate of evaporation, and to reduce the effects of variation in osmotic potential on results.

Spores were imbibed in the dark at 10°C for 24 hours prior to sowing. Spores were sown using an Eppendorf model 4810 adjustable (0.5–10 μl) pipettor and Eppendorf GELoader tips with the ends cut to fit the size of imbibed spores. Differences in the relative sizes of the paired spores were noted if they were apparent as observed through a Bausch and Lomb dissecting microscope at 30× magnification without the aid of a measuring device. Inoculated plates

* Author for correspondence

were placed into large fiberglass containers, which were covered with plastic wrap and placed on a light bench with continuous florescent and incandescent light.

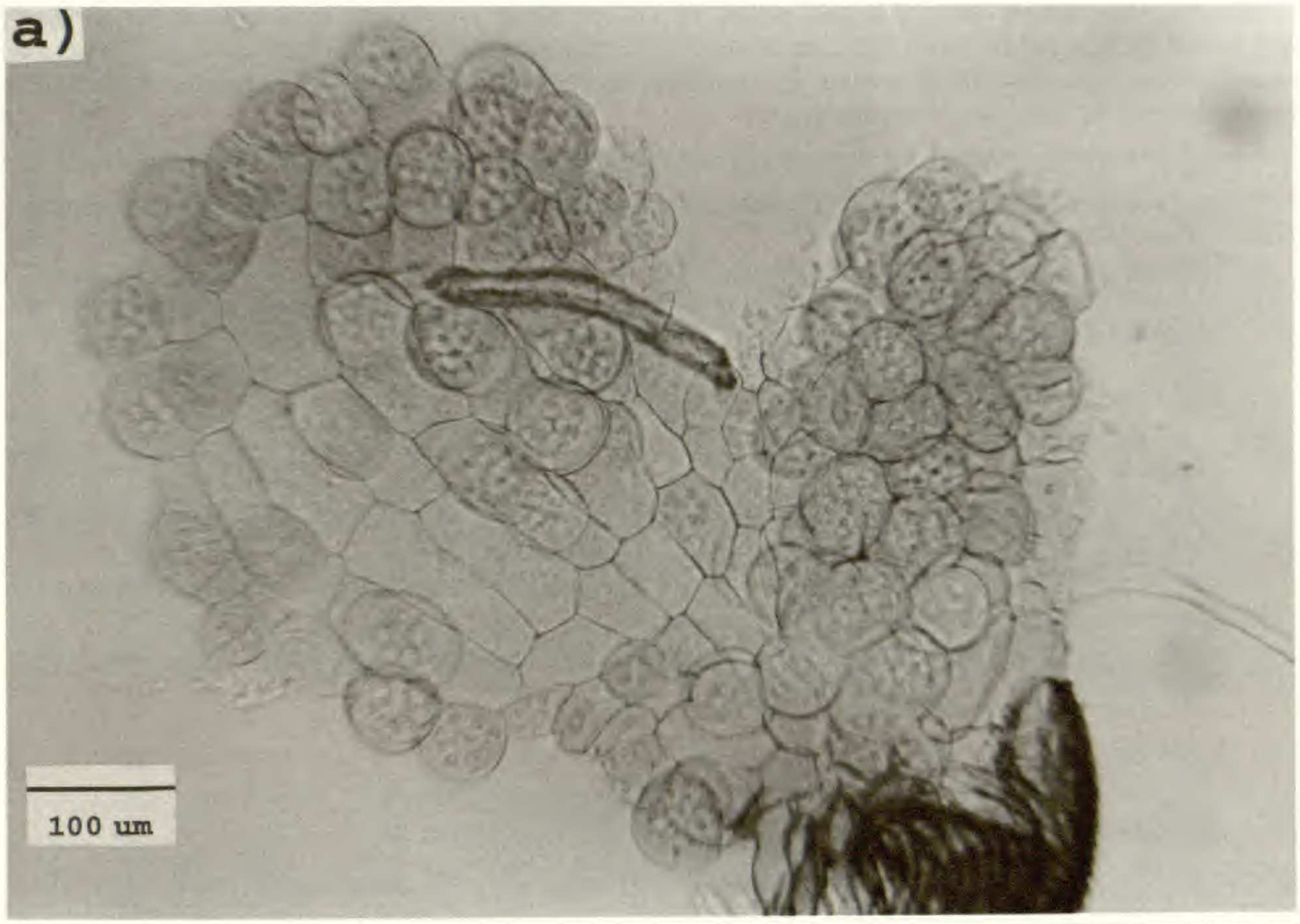
The experimental and control treatments were both prepared on June 7, 1994. Spore germination was observed 8:00AM, 2:00PM and 10:00PM each day from June 9 until the spores germinated. Spore germination was observed as both rupture of the spore wall and the appearance of a rhizoid. Gametophytes were harvested on June 22, 1994, and placed into a drop of chloral hydrate on a microscope slide, with observations made of the length and width of each gametophyte, along with the presence of antheridia or archegonia, using a Wild microscope. A Nikon AFM photomicrographic attachment was used for photography. The size of each gametophyte was estimated by using its length and width to calculate the area of an ellipse ($WL\pi/4$).

Results

Spore germination was first observed at 10:00 PM on June 9, and all spores had germinated by 2:00 PM on June 11 (Table 1). All control gametophytes were large hermaphrodites except for #25, which was archegoniate only, and #26 which was a small male. Several spores in both the treatment and the control germinated, but did not develop further, and several spores/gametophytes died between germination and harvest.

The mean size of hermaphroditic gametophytes in single spore cultures was 2.66 mm^2 (s=0.88; n=21), and 2.71 mm^2 (s=0.99; n=37) in paired spore cultures. The mean size of male gametophytes in paired spore cultures was 0.18 mm^2 (s=0.09; n=12). The male gametophyte in the single spore culture was developmentally similar to some of the male gametophytes in the paired spore cultures, with the exception that it had only one antheridium and a meristic notch was either present or forming (Fig. 1b). Male gametophytes in paired spore cultures had numerous antheridia and no evidence of a meristem in the notch region, if a notch was present (Fig. 1a).

Both spores developed in 24 of the 30 paired spore cultures. Each gametophyte pair was either hermaphrodite/hermaphrodite (H/H), hermaphrodite/male (H/M), or male/male (M/M) (Table 1). Among the 24 gametophyte pairs, 23 developed from spore pairs where spore sizes were different, resulting in 11 H/M, 11 H/H and 1 M/M pairs. Among the 10 pairs of different sized spores that germinated at the same time there were 1 M/M, 1 H/M and 8 H/H pairs. Among the 11 pairs where the larger spore germinated first, 9 developed into H/M pairs and 2 developed into H/H pairs. Twice the smaller spore germinated before the larger spore (sample #21 and #25), with one such pair developing into an H/H and the other into an H/M pair. The larger spore in pair #25 developed into an hermaphrodite, despite the fact that it germinated later than the smaller spore. One pair of spores (#15) were the same size, germinated at the same time, and developed into an H/H pair.



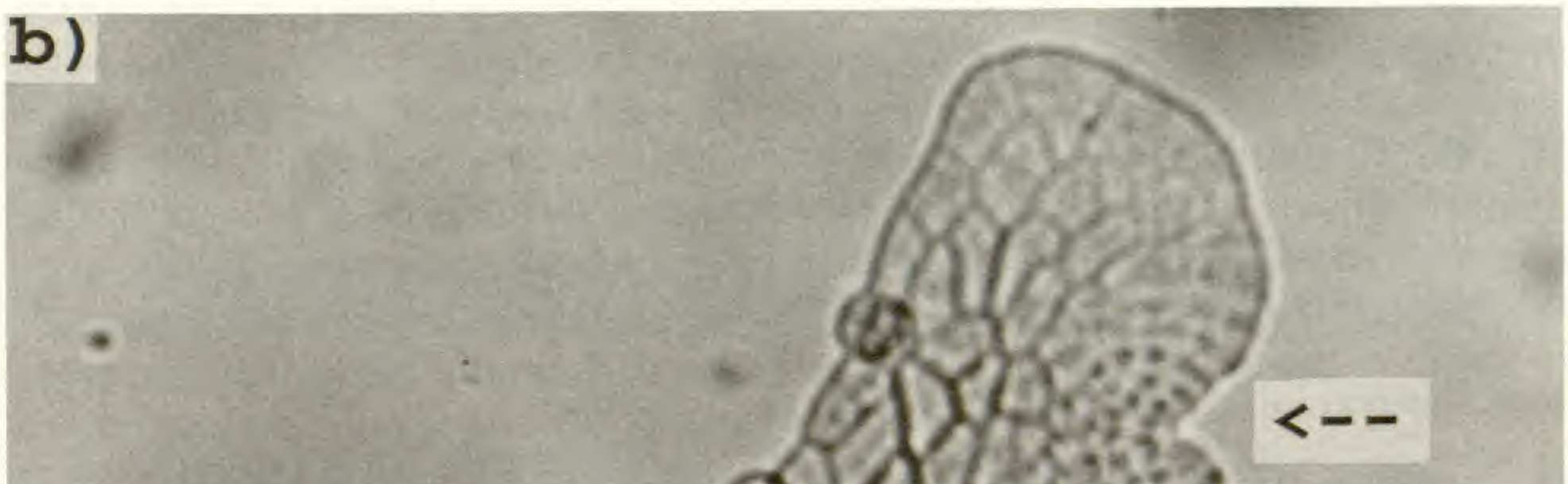


FIG. 1. Representative gametophytes: a) male gametophyte from paired culture #3; b) Male gametophyte from monospore culture #26. Arrow indicates meristic notch.

TABLE 1. Germination time, size, and gender of gametophytes developing from monospore (control) and paired (treatment) cultures. Sizes are in mm^2. ND indicates spores germinated, with no further gametophyte development. DIED indicates gametophyte died between germination and harvest. H indicates hermaphrodite, M male, and F female gender.

	Monospore			Paired			
Sample	Germination time	Size	Gender		Germination time	Size	Gender
1	11:08 am	2.83	H	a	10:10 pm (l)	1.67	H
				b	10:10 pm	1.67	H
2	11:08 am	3.57	H	a	10:10 pm (l)	1.83	H
				b	10:10 pm	1.83	H
3	11:08 am	1.48	H	a	11:02 pm	0.26	M
				b	10:10 pm (l)	1.38	H
4	11:02 pm	3.73	H	a	10:10 pm (l)	3.50	H
				b	10:10 pm (l)	4.00	H
5	11:08 am	2.32	H	a	9:10 pm (l)	5.60	H
				b	11:08 am	0.20	M
6	10:08 am		DIED	a	10:10 pm	1.62	H
				b	10:10 pm (l)	3.73	H
7	11:02 pm	1.40	H	a	10:10 pm	2.50	H
				b	10:10 pm (l)	3.00	H
8	10:10 pm	1.29	H	a	10:10 pm (l)	2.83	H
				b	10:10 pm	1.75	H
9	10:10 pm	2.68	H	a	11:08 am	0.04	M
				b	10:10 pm (l)	0.93	H
10	11:08 am	2.60	H	a	11:02 pm		ND
				b	11:02 pm (l)	2.06	H
11	10:10 pm		ND	a	11:08 am	3.31	H
				b	10:10 pm (l)	3.31	H
12	11:08 am	3.21	H	a	10:10 pm	0.50	H
				b	10:10 pm (l)	0.44	H
13	10:10 pm	2.86	H	a	11:02 pm		ND
				b	11:02 pm (l)	4.12	H
14	10:10 pm	4.09	H	a	11:08 am	0.27	M
				b	10:10 pm (l)	2.10	H
15	11:08 am	3.90	H	a	10:10 pm	3.20	H
				b	10:10 pm	2.85	H
16	11:08 am	2.57	H	a	10:10 pm (l)	2.70	H
				b	11:02 pm	0.30	M
17	10:10 pm	4.09	H	a	11:08 am	0.14	M
				b	10:10 pm (l)	4.12	H
18	10:10 pm	2.05	H	a	11:10 pm		ND
				b	10:10 pm	3.78	H
19	10:10 pm		DIED	a	11:02 pm (l)	3.25	H
				b	11:02 pm	0.23	M
20	11:08 am	2.80	H	a	11:08 am	3.25	H
				b	10:10 pm (l)	2.33	H
21	11:02 pm	1.70	H	a	11:08 am (l)	2.40	H
				b	10:10 pm	3.25	H
22	10:10 pm	2.29	H	a	11:08 am (l)	1.83	H
				b	11:02 pm	0.33	M
23	11:02 pm		ND	a	10:10 pm (l)	2.00	H
				b	10:10 pm		DIED

TABLE 1. Continued.

	Monospore			Paired			
Sample	Germination time	Size	Gender		Germination time	Size	Gender
24	10:10 pm		DIED	a	10:10 pm (l)		DIED
				b	10:10 pm		DIED
25	11:02 pm	0.80	F	a	11:08 am (l)	3.86	H
				b	10:10 pm	0.14	M
26	11:02 pm	0.06	M	a	11:02 pm		DIED
				b	11:02 pm (l)	1.36	H
27	11:08 am	2.72	H	a	11:08 am (l)	1.60	H
				b	11:08 am	1.50	H
28	11:08 am		DIED	a	10:10 pm	0.08	M
				b	10:10 pm (l)	0.09	M
29	11:02 pm		DIED	a	11:08 am	0.17	M
				b	10:10 pm (l)	1.92	H
30	10:10 pm	1.60	H	a	11:08 am	0.17	M
				b	10:10 pm (l)	3.34	H

DISCUSSION

Single spore cultures nearly always developed into large hermaphroditic gametophytes. Gametophytes in paired spore cultures were either male or hermaphroditic. Hermaphroditic gametophytes in paired cultures and monospore cultures were the same size. Male gametophytes in paired spore cultures were smaller than hermaphrodites. The only observed effects of pairing was the appearance of small male gametophytes in some cultures.

Males appeared most often when pairs of spores were of different sizes and the larger spore germinated first, with the smaller, later-germinating spore developing into a male gametophyte (9/11 H/M pairs). The greatest effect of neighbors was thus found among paired spore cultures where both spore size and germination time varied.

Small male gametophytes appeared in only 11 of 23 paired spore cultures where relative size differences existed. Spore size alone thus does not account for differences between paired and single spore cultures. This observation conflicts with Schedlbauer's (1976) conclusion that spore size alone had the greatest effect on gametophyte development. Schedelbauer studied the effects of meristic neighbors on newly germinated gametophytes. Any effects of early germinating spores on ungerminated neighboring spores, or effects of larger ameristic gametophytes on smaller ameristic gametophytes, were not present in Schedelbaur's experiment. Neighbor effects in our experiment could have begun immediately after imbibition, at least 2 days prior to spore germination. Given observations of the effect of dark spore germination in response to the presence of neighboring gametophytes (e.g., Schneller, 1979), pregermination effects of neighbors are known. The differences between our experiment and Schedlbauer's suggest that there may be effects of neighbors, other than dark germination, on very early gametophyte development.

Schedlbauer (1976) assumed that an antheridiogen was involved in regulating neighbor effects in his experiment. Evidence of the effects of antheridiogens is equivocal in our experiment. The small male in a single spore culture had one antheridium and a meristic region (Fig. 1b). The male gametophytes in the paired cultures had numerous antheridia and lacked a meristic region (Fig. 1a). Given the small size of the male gametophyte in the monospore culture, its development was hindered at least as much as among males in paired cultures, which strongly suggests that retardation of development alone is not enough to invoke an "antheridiogen response" in *C. richardii.*

The presence of a paired culture with 2 male gametophytes, similar to other males in paired cultures, conflicts with the classic antheridiogen hypothesis (as stated in Näf et al., 1975). The classic antheridiogen hypothesis suggests that antheridia are induced by the secretions of meristic gametophytes. This cannot account for the M/M pair. It is also difficult to apply the classic antheridiogen hypothesis as an explanation for H/M cultures, as the oldest gametophyte in such cultures was 13 days old. Although there was no accounting of time of formation of the notch and the meristem, it is unlikely that they were present long enough to account for the H/M pairs observed. The notch and meristem definitely did not form during the time interval between germination of spore pairs that germinated at different times. Given the great differences in size and state between males and hermaphrodites in H/M pairs, it is highly unlikely that development of the two gametophytes began to diverge only after the formation of a meristem in one of the gametophytes. Intergametophytic interactions leading to an antheridiogen response likely began much earlier in development.

Alternatively, if a larger earlier germinating spore could deprive a smaller, later-germinating spore of some essential resource, then the appearance of small males in paired spore cultures could be due to competition alone. Such an effect could occur as a result of differences in spore size and germination time. Such an effect could explain the differences in morphology between the male in monospore culture and the males in paired cultures. Earlier germinating spores, when developing at a sufficiently rapid rate, could reduce local resource availability, affecting the development of other gametophytes.

For *C. richardii* at least, the "antheridiogen response" is the result of intergametophytic actions beginning very early in gametophyte development. Intergametophytic effects on development occur most often when both spore size and spore germination time vary. Further studies of the effects of the wide range of factors that can affect gametophyte development in natural populations are needed to fully resolve the mechanisms by which gametophyte development is regulated.

Literature Cited

Klekowski, E. J. 1969. Reproductive biology if the Pteridophyta. III. A study of the Blechnaceae. Bot. J. Linn. Soc. 62:361–377.

Näf, U., K. Nakashini, and M. Endo. 1975. On the physiology and chemistry of fern antheridiogens. Bot. Rev. 41:315–359.

SCHEDLBAUER, M. D. 1976. Fern gametophyte development: controls of dimorphism in *Ceratopteris thalictriodes*. Amer. J. Bot. 63:1080–1087.

SCHNELLER, J. J. 1979. Biosystematic investigations on the lady fern (*Athyrium filix-femina*). Pl. Syst. Evol. 132:255–277.

SILVERTOWN, J. W. 1987. *Introduction to plant population ecology*, edition 2. Wiley, New York.